cells are returned to the host, subject and/or donor providing a system in which a particular target protein can now be readily induced to target cells for example orally by taking a pill of antibiotic rifampicin, or by suitable administration to cells of any compound recognized by the xenobiotic receptor as a ligand.

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[0026] Figure 1 collectively illustrates that SXR is a novel orphan nuclear receptor.

[0027] Figures 1A & 1B shows the sequence of the longest SXR cDNA clone (SEQ ID NO: 1) and a corresponding encoded protein (amino acids 41-434 of SEQ ID NO: 2). The DNA binding domain (amino acids 41-107) is shown in bold, and upstream termination codons in frame with the putative initiator leucine are indicated by asterisks. That this Leu can function as an initiator was demonstrated by SDS-PAGE analysis of labeled proteins produced from *in vitro* transcribed, translated cDNAs. The unmodified cDNAs yielded a translation product indistinguishable in size from that produced when the leucine was changed to methionine, albeit not nearly as efficient.

[0028] Figure [1B] 1C presents a schematic comparison between SXR and other members of the steroid hormone receptor super family such as e.g., RXR partners, the *Xenopus* benzoate X receptor (xBXR), the human vitamin D3 receptor (hVDR), the human constitutively active receptor-alpha (hCARα), the rat farnesoid X receptor (rFXR), the human peroxisome proliferator activated receptor alpha (hPPARα), the human liver-derived receptor X (LXRα), the human retinoic acid receptor alpha-1 (hRARα-1), the human thyroid hormone receptor beta (hTRβ), the human retinoid X receptor alpha (RXRα) and the human glucocorticoid receptor alpha (hGRα)). Ligand-binding domain boundaries follow those for the canonical nuclear receptor ligand-

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[0043] **Figure 9** presents schematic representations of the Alb-SXR and Alb-VPSXR transgene constructs. The filled region, stippled region, open region, and the crosshatched region, correspond to the mouse albumin promoter/enhancer, the xenopus β-globin leader and trailer sequences, the cDNAs of the wild type (SXR) or an activated form of SXR (VPSXR, with the fusion of VP16 activation domain at the 5' end as depicted), and the SV40 sequence containing the poly (A) processing signal, respectively.

Example 4

DNA-binding analysis

[00147] Electrophoretic mobility shift assays were performed using *in vitro* transcribed, translated proteins (TNT, Promega). Proteins (1 µl each) were incubated for 20 minutes at room temperature with 100,000 cpm of Klenow-labeled probes in 10 mM Tris pH 8, 100 mM KCl, 6% glycerol, 0.05% NP-40, 1 mM dithiothreitol (DTT), 100 ng/µl poly dI:dC (Pharmacia, Piscataway, NJ) and then electrophoresed through a 5% polyacrylamide gel in 0.5x TBE (45mM Tris-base, 45 mM boric acid, 1 mM ethylenediaminetetraacetic acid (EDTA) at room temperature. For competition binding, protein plus unlabeled oligonucleotides at five or fifty fold molar excess were preincubated for ten minutes on ice, then labeled probes were added and incubated for 20 minutes at room temperature. Electrophoresis was as above. The IR series oligonucleotides tested had the following sequences:

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IR-M, agcttACGTCATGACGTa (SEQ ID NO:32).

Mutations in the IR-M nucleotide sequence prevented binding of the heterodimer to the response element.

CYP3A oligonucleotides tested had the following sequences:
CYP3A4, tagaataTGAACTcaaaggAGGTCAgtgagtgg (SEQ ID NO: [31] 33);

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Example 5

Plasmid constructs and mutagenesis

[00148] The CYP3A23 cellular promoter reporter, PGL3-CYP3A23, was cloned by inserting the PCR-amplified 5' regulatory sequence of rat CYP3A23 gene (nt -1360 to 82) (Burger, *et al.* 1992) into the PGL3 vector (Promega). PGL3-CYP3A4 contains up to nt -1093 of the 5' flanking regions of the human CYP3A4 gene (Hashimoto *et al.*, 1993). Site-directed mutagenesis was performed by the PCR overextension method (Ho *et al.*, 1989). The PCR-amplified sequences and target mutagenesis were confirmed by DNA sequencing.

[00149] The expression vectors for the wild type SXR (pCDG-HX7), an activated form of SXR (pVPG-HX7), and the wild type PXR (pCDG-PXR) were described previously (Blumberg et al, 1998).

Example 6

Preparation of hepatocytes, DNA transfections and drug treatment

[00150] Primary cultures of rat hepatocytes were prepared as described previously (Li et al, 1991, and Barwick, *et al.* 1996). Lipofectin (Gibco-BRL)-mediated DNA transfections were carried out as described (Barwick, *et al.* 1996). When necessary, cell were treated with RIF, DEX, PCN, nifedipine, CTZ, corticosterone, coumestrol, RU486, cortisol, 17β-estrodiol (E2),

CYP3A4 can be activated by the rodent-specific activator PCN when the promoter was introduced into the rodent cellular environment, presumably via the activation of the endogenous PXR; on the other hand, RIF can active the CYP3A4 in the rodent cellular environment with the introduction of human SXR. The SXR-mediated activation of CYP3A23 or CYP3A4 cellular promoter by RIF exhibited dose dependence of both receptor and ligand (data not shown).

[00153] The fact that SXR is necessary and sufficient to render the induction of both human CYP3A4 and rat CYP3A23 gene in rodent hepatocytes by RIF suggested that the host cellular environment, SXR/PXR herein, rather than the gene structure, dictates the patterns of inducibility of CYP3A genes. The above notion would predict: (1) The SXR/PXR response element is essential for the activation of CYP3A genes; and (2) The response elements of SXR and PXR are interchangeable. Therefore, mutagenesis analysis was performed on the promoter of the rat CYP3A23 gene to examine these predictions. In vitro electrophoretic mobility shift assays showed that both SXR:RXR and PXR:RXR heterodimers efficiently bind to the DR-3 element (5' TGAACTtcaTGAACT 3') (SEQ ID NO: 39) in the CYP3A23 promoter (Blumberg *et al.*, 1998, 1998). As shown in Figure 8C, mutation of both half sites (DR3/M1) or a single half site (DR3/M2) abolished the PXR and/or SXR-mediated activation by PCN, RIF, and CTZ; On the other hand, replacement of the wild type DR-3 element by an IR-6 element of the human CYP3A4 gene promoter (Blumberg *et al.*, 1998, and Kliewer *et al.*, 1998) successfully rescueS the inducibility by PCN, RIF and CTZ.

[00154] Taken together, the transfection results demonstrate that nuclear receptors SXR/PXR are essential in determining patterns of CYP3A inducibility. In addition, these results establish successful development of a cell culture system allowing trans-species gene transfer and CYP3A inducibility.

Example 8

Generation and identification of transgenic mice

[00155] To generate Alb-SXR and Alb-VPSXR transgenes, the SXR and VPSXR cDNA were released from pCDG-HX7 and pVPG-HX7 (Blumberg *et al.*, 1998), and cloned into the Bam HI site downstream of the mouse albumin promoter/enhancer (Pinkert *et al.*, 1987), respectively. A SV40 intron/poly (A) sequence (Xie *et al.*, 1999) was subsequently placed downstream of SXR and VPSXR cDNAs. The 8.45 kb Alb-SXR, and 8.75 kb Alb-VPSXR transgenes were excised from the vector via Not I and Asp 718 digestion, and purified from agarose gel using QIAquick Gel Extraction Kit (QIAGEN). Microinjection of transgene into one-cell CB6F1 mouse zygotes was carried out at the Salk Institute Transgenic Animal Facility. All mice were handled in an accredited Institute facility in accordance with the institutional animal care policies.

[00156] Genomic DNA was isolated as described before (Xie *et al.* 1999). The polymerase chain reaction (PCR) was used to screen the transgene positive mice. Two oligonucleotides used to screen Alb-SXR mice are 5'-GAGCAATTCGCCATTACTCTGAAGT-3' (SEQ ID NO: 36) (annealing to SXR cDNA), and 5'-GTCCTTGGGGTCTTCTACCTTTCTC-3' (SEQ ID NO: 37) (annealing to the SV40 sequence downstream of the transgene in the transgene cassette). Another two oligonucleotides used to screen Alb-VPSXR are 5'-GACGATTTGGATCTGGACATGTTGG-3' (SEQ ID NO: 38) (annealing to VP16 sequences), and 5'-GTTTTCATCTGAGCGTCCATCAGCT-3' (SEQ ID NO: 40) (annealing to the SXR cDNA). PCR was carried out in a DNA thermal cycler (Perkin-Elmer/Cetus) using the following program: 94 °C for 1 min, 58 °C for 2 min, and 72 °C for 3 min and products were analyzed by electrophoresis on a 1% agarose gel. The transgene integration status was analyzed by Southern blot using transgene specific probes as described before (Xie *et al.* 1999).

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